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Plasmid-mediated horizontal gene transfer is a coevolutionary process

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Abstract

Conjugative plasmids are key agents of horizontal gene transfer that accelerate bacterial adaptation by vectoring ecologically important traits between strains and species. However, while many conjugative plasmids carry beneficial traits, all plasmids exert physiological costs-of-carriage on bacteria. The existence of conjugative plasmids therefore presents a paradox, since non-beneficial plasmids should be lost to purifying selection, whereas beneficial genes carried on plasmids should be integrated into the bacterial chromosome. Several ecological solutions to the paradox have been proposed, but none account for coadaptation of bacteria and conjugative plasmids. Drawing upon evidence from experimental evolution, we argue that horizontal gene transfer via conjugation can only be fully understood in a coevolutionary framework.

Mechanisms of horizontal gene transfer

Horizontal gene transfer (HGT) is a major process in the evolution of bacteria. The uptake of ready-made genes or operons from the 'mobile gene pool' facilitates rapid adaptation to novel environments, without the reliance upon rare, beneficial mutations arising spontaneously in the population [1]. As such, HGT is often associated with evolutionary and ecological innovation, conferring new phenotypic traits (or suites of traits) and thereby access to novel ecological niches [2, 3]. The effectiveness of this mode of adaptation is acutely demonstrated by the rapid global spread of antibiotic resistance throughout bacterial populations [4]. Importantly, because HGT can occur between taxonomically distinct bacterial lineages, and even between kingdoms [5], it blurs the boundaries between clades and obscures phylogenetic relationships. Yet conversely, since species-specific traits, i.e. those that distinguish sister clades, often

arise through HGT, it is equally an important driver of bacterial speciation [2, 3]. As a consequence of HGT, microbial diversity should be viewed less as a reticulate tree, and more as a thicket of interconnecting branches [6].

HGT is mediated by three different mechanisms: **transformation, transduction** and **conjugation** (for items in bold see glossary) [7]. It is curious that despite HGT underpinning bacterial adaptation, only one of these mechanisms, transformation, is under the control of bacteria. Both transduction and conjugation are mediated by semi-autonomous vectors: temperate phages and conjugative elements respectively (of which conjugative plasmids are the most significant) [7]. Because these vectors encode genes controlling their own replication and transmission they must be considered as evolving agents subject to natural selection in their own right, with fitness interests that need not necessarily be aligned with those of their bacterial host. There is therefore opportunity for both conflict and collaboration between bacteria and HGT vectors, generating reciprocal selection and thus the potential for on-going adaptation and counter-adaptation. In this essay, we argue that to better understand vector mediated HGT, a coevolutionary rather than simply evolutionary approach should be taken. We focus on conjugative plasmids, for which a large body of theory has been developed to understand their population biology and identify the ecological conditions for their maintenance.

The plasmid paradox

Conjugative plasmids are a diverse group of (mostly) circularized DNA molecules that exist independently of the host bacterial genome. Plasmid genomes consist of a backbone containing essential genes controlling core plasmid functions as well as a suite of non-essential accessory genes [Box 1]. It is these accessory genes that provide the currency of HGT, encoding traits that are potentially beneficial to the bacterial host. Accessory genes can be divided into three key functional groups: those conferring virulence, by allowing their hosts to inhabit and exploit other organisms [8], resistance to toxins such as antibiotics [9] and heavy metals [10], and metabolic functions such as nitrogen fixation in rhizobia [11]. It is notable that many accessory gene encoded traits are expressed outside of the cell, i.e. the gene products are secreted, thereby leading to the hypothesis that HGT may play a key role in microbial sociality [12]. Accessory genes are themselves often carried on smaller mobile

elements embedded within the plasmid [10, 13], allowing them to mobilize within and between plasmids, as well as integrate into the host chromosome.

A great deal of attention has been focused on establishing the theoretical 'existence conditions' for conjugative plasmids [14-17]. The carriage of plasmids exerts a high physiological burden on the host cell. The upkeep and repair of plasmid DNA [18] and the production of plasmid proteins [19] uses up raw materials within the cell, occupies cellular machinery such as ribosomes [18] and disrupts the cellular environment [20]. In addition to being energetically costly, production of conjugative pili also exposes the cell to attack from pilus-specific bacteriophage [21]. **Positive selection** for beneficial, plasmid-borne accessory traits could outweigh this cost. However, consistent positive selection on beneficial traits is predicted to ultimately favor the integration of these traits into the host chromosome and the subsequent loss of the plasmid backbone [15]; a process facilitated by the location of accessory genes on mobilizable elements within the plasmid genome. In the absence of positive selection, conjugative plasmids are predicted to be lost from the population by **purifying selection** unless plasmids are capable of very high rates of conjugative transfer [15, 22]. Whether such rates are achievable in nature has been hotly debated [16, 22, 23]. Moreover, plasmids persisting through conjugation alone would be expected to experience strong selection to jettison extraneous genetic material including their complement of accessory genes [24].

Explaining the existence and ecological persistence of beneficial conjugative plasmids therefore presents a paradox: in the absence of positive selection, highly conjugative plasmids should evolve high transmission rates and lose their accessory genes, whereas under consistent positive selection beneficial accessory traits should be integrated into the bacterial chromosome. How then is the rich diversity of plasmid vectors and their accessory elements maintained? A number of long term bacteria–plasmid co-culture experimental evolution studies (summarized in Table 1) provide a test-bed for theoretical predictions.

Resolving the plasmid paradox: a role for coevolution?

A consistent finding across co-culture studies is that costly plasmids are not easily lost from bacterial populations, and can be maintained for hundreds of generations, even

in the absence of positive selection [24-29]. This pattern cannot be accounted for by high conjugation rates alone, because non-conjugative plasmids are also maintained over these long timescales [24, 26, 27, 29]. Nor can this pattern be explained by stringent segregation systems, such as post-segregational killing mechanisms, as these were lacking in several studies [24, 26]. The surprising stability of bacteria-plasmid associations can be attributed to evolutionary adaptation. In the vast majority of long-term co-culture experiments, persistence is associated with a reduction in the burden of plasmid carriage [24-27, 29-34] (although notable exceptions exist [28]). This weakens the strength of purifying selection against plasmid carriage, and therefore reduces the rate at which plasmids are removed from the population.

A number of co-culture studies have attempted to determine the extent to which co-adaptation of both bacteria and plasmid, rather than simply adaptation by one party or the other, contributes to higher than expected plasmid stability [24-26, 30, 32]. By comparing costs-of-carriage between evolved and ancestral plasmids in both evolved and ancestral host genetic backgrounds, the relative contributions of bacterial and plasmid evolution can be deduced [Box 2]. Reduction in costs-of-carriage could, in 4 of the 5 studies, be attributed to coadaptation, with both host and plasmid adaptations contributing to improved fitness [24-26, 32]. For example, following 1100 generations without positive selection for plasmid-encoded traits, Dahlberg & Chao [25] observed, in 5 of 6 evolved bacteria-plasmid clones, complete amelioration of the cost-of-carriage, i.e. no difference in fitness was detected between evolved bacteria with or without their co-evolved plasmid. Further assays measuring the fitness of constructed bacteria-plasmid clones suggest that improved fitness resulted from adaptations by both bacteria and plasmids: reduced costs-of-carriage were observed for evolved plasmids in the ancestral genetic background (indicating plasmid adaptation), and for the ancestral plasmid in the evolved bacterial genetic background (indicating bacterial adaptation).

Mechanisms of amelioration

Co-culture studies therefore suggest that bacteria-plasmid coadaptation could broaden the conditions favouring plasmid persistence. Such studies highlight 3 key mechanisms by which amelioration can occur: changes in conjugation rate, loss of plasmid genes and changes in plasmid gene expression.

Conjugation rate

Dahlberg & Chao [25] observed that in two populations, evolved plasmids entirely lost the ability to conjugate, while another population had a reduced conjugation rate associated with the evolution of suppression by the bacterial host. Conjugation is thought to impose a cost to the host, which must invest energy in pili formation and plasmid replication [34], thus a positive relationship is expected between the cost-of-carriage and conjugation rates. Such a correlation has been demonstrated by Turner *et al.* [34] who found that plasmids which evolved lower conjugation rates imposed lower fitness costs in the ancestral bacterial background, while those that had evolved increased conjugation rates imposed greater costs. Reduced conjugation rates represent a shift towards higher investment in vertical transmission, and thereby closer alignment of bacterial and plasmid fitness interests, because plasmid fitness is more dependent upon bacterial growth rate. These findings stand in stark contrast to theoretical predictions that plasmid maintenace in the absence of positive selection requires high conjugation rates [22]. The evolution of reduced conjugation rates however suggests that co-adaptation may lead to the domestication of plasmid genomes and a reduction in HGT.

Loss of plasmid genes

Amelioration of the cost-of-carriage may also be achieved through the loss of the non-essential portion of the plasmid genome. When not under positive selection, accessory genes represent 'excess baggage'; increasing the number of genes requiring transcription and translation by the host [24]. The loss of accessory genes has been found to occur during co-culture, and has been shown to lead to a reduced cost-of-carriage [24]. In one case, amelioration by the plasmid was due to a large deletion, encompassing ¼ of the plasmid genome as well as a tetracycline resistance cassette [24]. Large deletion events can therefore be a rapid route to amelioration of the cost-of-carriage, but the loss of accessory traits from the population would ultimately negate the role of plasmids in HGT. However, co-culture studies also demonstrate that, like their plasmid vectors, accessory traits are not easily lost. Interestingly, in the same study, an ampicillin resistance marker was maintained in the absence of selection [24]. This difference is likely to be due to the deleted region corresponding to a mobile **integron**, which was therefore more easily excised. Dahlberg & Chao

[25] note that although plasmids lacking antibiotic resistance markers did arise in experimental populations, they remained at low frequencies through out the experiment. A longer-term study, following four different multi-drug resistant plasmids in *Escherichia coli* found that antibiotic resistance was maintained for between 500 to 1000 generations before genes conferring resistance to different antibiotics were gradually lost [27]. Therefore accessory gene loss appears to be unexpectedly rare. Where it does occur, the association of loss events with mobile elements may allow retention of such genes within the wider mobile gene pool, simply because those accessory genes most likely to be excised are also those most likely to integrate elsewhere.

Reduced gene expression

Gene expression represents a key cost of carrying additional DNA [35-37]; therefore down regulation of plasmid genes could play a role in amelioration. Transcription is also likely to present a target for host associated amelioration, as bacteria are able to exert control over plasmid gene expression [38], potentially stabilizing bacteria-plasmid associations [39]. Only a single study has investigated the effect of long term co-culture on plasmid gene expression [33]. Heuer et al. [33] allowed an antibiotic resistance plasmid to evolve over 1000 bacterial generations in populations of *Pseudomonas putida*, under a regime in which the plasmid was switched regularly between host strains. Following 1000 generations under antibiotic selection the cost of carriage was reduced. Plasmid core genes, including those involved in conjugation and stability, as well as some accessory genes were down-regulated. Conversely, plasmid-borne antibiotic resistance genes that were under positive selection were expressed at a higher level in coevolved bacteria-plasmid clones. Changes in gene expression are likely to be important for HGT, as reduced expression lowers the costs associated with accessory genes while allowing their retention and thereby their maintenance in the population.

Specificity of coadaptation

Following long-term co-culture of a conjugative R1 plasmid in *E. coli* under positive antibiotic selection, Dionisio *et al.* [32] observed that evolved plasmids ameliorated the cost-of-carriage in all populations. Indeed, plasmids from two populations, when placed into the ancestral bacterial genetic background, actually increased bacterial

fitness relative to plasmid-free cells. Surprisingly, this amelioration was maintained even when evolved plasmids were placed into a naïve *Salmonella* strain [32]. The mechanism underlying this fitness increase is uncertain, but demonstrates the potential for generalized plasmid adaptations, whereby adaptations evolved in one host background can confer improved fitness in alternative hosts. Similar findings have been reported in studies specifically selecting on plasmid host range. De Gelder *et al.* [31] show that adaptation of a conjugative plasmid to a novel host (under positive antibiotic selection) resulted in an expansion of host range, ameliorating the cost of plasmid carriage in both the ancestral host, as well as a second, naïve novel host species. A further study demonstrates that regular switching of bacterial host species resulted in greater amelioration in the ancestral background, relative to plasmids co-cultured with a single host species [33].

Generalist plasmid adaptations are not, however, consistently observed across studies. Modi & Adams [24] describe one evolved plasmid genotype which imposed a smaller burden on its coevolved host, but when returned to its ancestral host, imposed a significantly greater burden than the ancestral plasmid [26]. This illustrates the potential for evolution of specialized coadaptation between host and plasmids, as opposed to more generalist adaptation observed by Dahlberg & Chao [25], where adaptations in the evolved plasmid improved fitness in both the evolved and ancestral bacterial genetic backgrounds. Understanding what drives the evolution of plasmid specificity will be important in predicting the fate of plasmids in bacterial communities, and the taxonomic breadth of HGT between strains and species via conjugation.

Integration of beneficial genes into the bacterial genome

Under consistent positive selection for plasmid borne traits, theory predicts that accessory genes will be integrated into the host chromosome [15]. This outcome has been reported in just one co-culture study. Modi *et al.* (1992) [29] observed chromosomal integration of a previously plasmid bound ampicillin resistance marker, located on a Tn3 transposon, in two independent populations. However, contrary to theory, this occurred in populations grown in the absence of ampicillin, and therefore not as a consequence of positive antibiotic selection. The absence of integration events in studies conducted under positive selection indicates that this is perhaps not

as widespread a response to selection as predicted [30-34, 40], at least not under laboratory conditions.

Virulent plasmids: the potential for reciprocal antagonism

It should be noted, that coevolution does not always tend towards amelioration of plasmid burden. In one study, plasmid-bacteria coevolution appeared to be highly antagonistic under conditions in which multiple plasmids were able to co-infect bacterial hosts [28]. The resulting within-host competition drove the evolution of extreme virulence in evolved plasmids when moved into their ancestral hosts, such that evolved plasmids were lethal in some instances. Reciprocal counter adaptations were observed in evolved bacterial populations which showed evidence of evolved resistance to plasmid infection, indicating the potential for antagonistic 'arms race' coevolution between plasmids and their hosts.

Concluding remarks

Co-culture studies have demonstrated that coadaptation has a major role to play in explaining the maintance of plamids and their accessory genes in bacterial populations. Under laboratory conditions, coevolution frequently leads to the amelioration of plasmid burden and consequently significantly broadens the range of ecological conditions favoring plasmid persistence. The evolution of generalist plasmids with improved fitness across a range of bacterial genetic backgrounds in some studies suggests that coevolution can potentially enhance the success of subsequent HGT event. Conversely, often the mechanisms underlying amelioration, such as reduced conjugation rate or accessory gene loss, suggest a shift towards vertical transmission and domestication, and therefore potentially reduced rates of HGT. Understanding the interaction between coadaptation and HGT requires future studies to explore a much wider range of ecological conditions to identify those factors that favour and those that counteract plasmid domestication (see box 3). Crucially, to date co-culture studies have largely focused on pairwise bacteria–plasmid associations under constant laboratory conditions, while in nature HGT occurs in much more complex environmental and community contexts. Several theoretical models explore the effects of heterogenous environments [9], spatial sturcture [17] and population dynamics [15] on plasmid persistence. However, these models ignore the potential role of co-adaptation. In order to properly understand the

fate of conjugative plasmids and their role in HGT, future theoretical and empirical work (Box 3) should be directed at bridging this gap.

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Glossary

Purifying selection: this acts to remove deleterious alleles from the population.

Positive selection: this acts to increase the frequency of beneficial alleles in the population.

Transformation: is the uptake of DNA from the environment by bacteria.

Transduction: is the transfer of DNA between cells via a phage vector.

Conjugation: is the transfer of DNA by direct cell-to-cell contact often mediated by conjugative plasmids.

Integron: a mobile genetic element carrying an integrase, which allows acquisition (or loss) of genes by homologous recombination.

Plasmid type[1]	Selection for plasmid borne traits?	Bacterial Generations	Change in cost of carriage[2]	Which party adapted?[3]	Study
Pairwise host – plasmid co-culture					
C	No	1100	⇓	c	[25]
N	No	650	⇓	c	[24]
N	No	773	- n/a (plasmid lost) -		[29]
N	No	773	⇓	c & p	[26]
N	Yes	500	⇓	b	[30]
C	Yes	420	⇓	c	[32]
Multihost–single plasmid co-culture					
C	Yes	1000	⇓		[33]
C	Yes	500	⇓		[31]
N	Yes	1000			[40]
Long term persistence					
C & N	No	4000			[27]
Within-host competition of coinfecting plasmids					
C	No	400	⇑		[28]
Enforcing horizontal and or vertical modes of plasmid transmission					
C	Yes	500	⇓		[34]

Table 1. Summary of co-culture studies and their outcomes

[1] Conjugating (C) or non-conjugating (N)
[2] '⇓' denotes a reduction in the cost-of-carriage, '⇑' denotes an increase
[3] 'c' denotes coevolution, 'p' denotes plasmid evolution and 'b' denotes

1

2

Box 1. What makes a plasmid?

Plasmid genomes are modular in structure, such that genes are broadly arranged into discreet operons encoding specific functions [41]. This structure is a consequence of frequent genetic recombination, forming a mosaic of genes from different sources. Plasmids can be subdivided into a core 'backbone' of genes encoding plasmid functions, and 'accessory' genes encoding traits beneficial to the bacterial host (discussed in the main text). 'Backbone' genes encode the following key functions: replication, segregation and conjugation.

Replication is the only function required to meet the basic definition of a plasmid. The replication region generally consists of an origin of replication (*ori*) as well as proteins that recruit the host's own DNA replication machinery (i.e. polymerase molecules, tRNAs and ribosomes) to carry out replication. Genes regulating plasmid replication are also common on plasmids, to ensure that the number of plasmid copies in the host remains stable.

Segregation systems act to minimise the loss of the plasmid during cell division. High copy number plasmids often lack such systems and rely on diffusion to ensure plasmids are present in both mother and daughter cells. However low copy plasmids often take a proactive approach to minimise mis-segregation. Active partitioning (*par*) systems mimic the mitotic process. Plasmids encode proteins that bind to a centromere-like region and direct plasmid molecules towards the poles of the dividing cell. Alongside this, many plasmids also utilise post-segregational killing. These encode a toxin-antitoxin system producing a stable toxin and a less stable antitoxin molecule: if the plasmid is lost, the antitoxin degrades quicker than the toxin in the cell, leading to cell death.

Conjugation genes allow the plasmid to transmit horizontally though cell-to-cell transfer. Conjugative plasmids encode genes for 'mate pair formation' – the formation of a physical link between donor and recipient cells, often in the form of a pilus. A second, sometimes separate, set of genes allows the one strand of the plasmid DNA to move into the recipient cell and become established [41]. Many 'mobilizable' plasmids forgo the need to carry their own mate pair formation genes however, piggybacking on the actions of coinfecting conjugative plasmids [41].

Box 2. Measuring coevolution

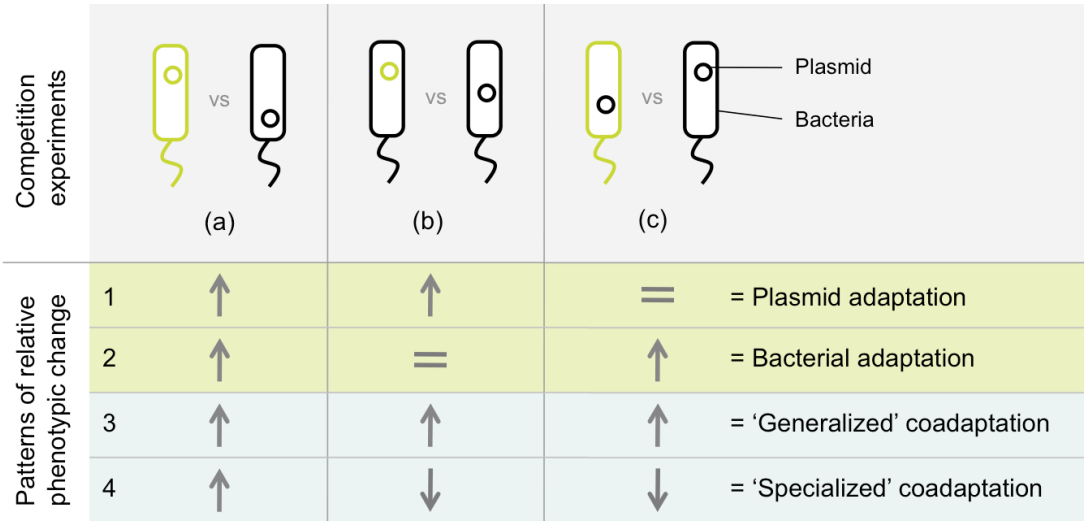

Figure I Coevolutionary changes can be detected through a series of comparisons between the different combinations of evolved (green) and ancestral (black) plasmid and bacteria, to the ancestral plasmid and bacteria. The pattern of change (arrows) and stasis (=) in fitness relative to the ancestor can be used to disentangle whether evolutionary or coevolutionary changes have occurred.

Coevolution can be inferred where changes in fitness (or other traits) are associated with adaptation in both plasmid and bacteria, following long-term co-culture. In figure I a series of competition experiments are shown in grey which can be used to unravel these interactions: (a) overall change is measured by competing the evolved (green) bacteria-plasmid against the ancestral (black) genotype, (b) adaptation in the plasmid is estimated by measuring fitness of the evolved plasmid in the ancestral background and (c) adaptation in the bacteria is measured by measuring fitness of the evolved bacteria carrying an ancestral plasmid.

Whether evolutionary or coevolutionary changes have occurred can then be inferred from the pattern of fitness change relative to the ancestor, where arrows denote change and = denotes no difference from ancestor. In Figure I, four hypothetical scenarios illustrate this point: (1) Where a difference is observed in comparisons (a) and (b), but not (c) this implies that no significant adaptation has occurred in the bacteria. Therefore the change is driven primarily by plasmid evolution. (2) In contrast, if no adaptation in the plasmid (b) is detected, this implies that the change is due to bacterial evolution. (3) If an increase in fitness is seen in all 3 comparisons, then this represents 'generalized' coadaptation, as adaptation has occurred in both plasmid and bacteria but is not specific to the coevolved partner. (4) If the change in

fitness in the coevolved bacteria-plasmid pair (a) is opposite to that measured in the plasmid (b) and bacteria (c) alone, this may indicate 'specialized' coevolution, as the increase in fitness is specific to the presence of the coevolved partner.

Box 3. Future directions

The genetic basis for coevolution: Deletion of sections of the plasmid genome – for instance, those encompassing accessory traits – is just one mechanism that plasmids can employ to reduce the physiological burden on the host. Selection can also focus on genes encoding core functions such as conjugation [34], segregation or more subtle changes such as reducing gene expression [33], which compensate for the presence of these additional genes. Understanding how frequently, and under what circumstances these different mechanisms occur will be an important step in understanding and predicting the fate of horizontally transmitted traits in microbial communities.

Coevolution in complex environments: Whether plasmids are beneficial or costly to their bacterial hosts is determined by the selective environment (e.g. the presence or absence of antibiotics). Heterogeneity in the direction of selection can theoretically favor the maintenance of beneficial traits on mobilizable plasmids [9], and such heterogeneity is predicted, by coevolutionary theory, to affect the maintenance of coadaptation across populations [42]. The interplay between ecological and evolutionary factors is likely to be crucial to understanding HGT in natural populations.

Coevolution in the meta-community: Many plasmids are promiscuous in terms of host range, and are likely to compete with other genetic elements with which they share hosts. Coevolution with multiple host species may impede adaptation to any given host because the intergenomic linkage between co-adapted genes will be continuously broken down. Competition and conflict with other mobile elements may drive greater antagonism between hosts and plasmids [28]. What impact therefore does community context have on bacteria-plasmid coevolution?

Levels of coevolutionary selection: The mobilizable elements on which beneficial accessory traits are themselves often located are likely to be subject to selection in their own right. HGT may therefore be a tripartite coevolutionary process between bacteria, conjugative plasmids and mobilizable elements; at what level reciprocal selection acts is likely to depend upon the environmental and community context.

1. Jain, R., *et al.* (2003) Horizontal gene transfer accelerates genome innovation and evolution. *Mol. Biol. Evol.* 20, 1598-1602
2. Ochman, H., *et al.* (2000) Lateral gene transfer and the nature of bacterial innovation. *Nature* 405, 299-304
3. Wiedenbeck, J., and Cohan, F.M. (2011) Origins of bacterial diversity through horizontal genetic transfer and adaptation to new ecological niches. *Fems Microbiol Rev* 35, 957-976
4. Bennett, P.M. (2008) Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. *Br J Pharmacol* 153, S347-S357
5. Heinemann, J.A., and Sprague, G.F. (1989) Bacterial conjugative plasmids mobilize DNA transfer between bacteria and yeast *Nature* 340, 205-209
6. Gogarten, J.P., and Townsend, J.P. (2005) Horizontal gene transfer, genome innovation and evolution. *Nat Rev Microbiol* 3, 679-687
7. Thomas, C.M., and Nielsen, K.M. (2005) Mechanisms of, and barriers to, horizontal gene transfer between bacteria. *Nature Reviews Microbiology* 3, 711-721
8. Elwell, L.P., and Shipley, P.L. (1980) Plasmid mediated factors associated with virulence of bacteria to animals. *Annu Rev Microbiol* 34, 465-496
9. Svara, F., and Rankin, D.J. (2011) The evolution of plasmid-carried antibiotic resistance. *BMC Evol Biol* 11, 10
10. Tett, A., *et al.* (2007) Sequence-based analysis of pQBR103; a representative of a unique, transferproficient mega plasmid resident in the microbial community of sugar beet. *Isme Journal* 1, 331-340
11. MercadoBlanco, J., and Toro, N. (1996) Plasmids in rhizobia: The role of nonsymbiotic plasmids. *Mol Plant-Microbe Interact* 9, 535-545
12. Rankin, D.J., *et al.* (2011) What traits are carried on mobile genetic elements, and why? *Heredity* 106, 1-10
13. Petrovski, S., and Stanisich, V.A. (2011) Embedded elements in the IncP beta plasmids R772 and R906 can be mobilized and can serve as a source of diverse and novel elements. *Microbiology-Sgm* 157, 1714-1725
14. Stewart, F.M., and Levin, B.R. (1977) Population Biology of Bacterial Plasmids - A Priori conditions for the existance of conjugationally transmitted factors. *Genetics* 87, 209-228
15. Bergstrom, C.T., *et al.* (2000) Natural selection, infectious transfer and the existence conditions for bacterial plasmids. *Genetics* 155, 1505-1519

16. Lili, L.N., *et al.* (2007) The persistence of parasitic plasmids. *Genetics* 177, 399-405
17. Krone, S.M., *et al.* (2007) Modelling the spatial dynamics of plasmid transfer and persistence. *Microbiology-Sgm* 153, 2803-2816
18. Glick, B.R. (1995) Metabolic load and heterologous gene expression. *Biotechnol Adv* 13, 247-261
19. Rozkov, A., *et al.* (2004) Characterization of the metabolic burden on Escherichia coli DH1 cells imposed by the presence of a plasmid containing a gene therapy sequence. *Biotechnol. Bioeng.* 88, 909-915
20. Ricci, J.C.D., and Hernandez, M.E. (2000) Plasmid effects on Escherichia coli metabolism. *Crit Rev Biotechnol* 20, 79-108
21. Jalasvuori, M., *et al.* (2011) Bacteriophage selection against a plasmid-encoded sex apparatus leads to the loss of antibiotic-resistance plasmids. *Biology Letters* 7, 902-905
22. Levin, B.R. (1993) The accessory genetic elements of bacteria: existence conditions and (co)evolution. *Curr Opin Genet Dev* 3, 849-854
23. Sorensen, S.J., *et al.* (2005) Studying plasmid horizontal transfer in situ: A critical review. *Nature Reviews Microbiology* 3, 700-710
24. Modi, R.I., *et al.* (1991) Plasmid macro evolution - selection of deletions during adaptation in a nutrient-limited environment. *Genetica* 84, 195-202
25. Dahlberg, C., and Chao, L. (2003) Amelioration of the cost of conjugative plasmid carriage in Eschericha coli K12. *Genetics* 165, 1641-1649
26. Modi, R.I., and Adams, J. (1991) Coevolution in Bacterial - Plasmid Populations. *Evolution* 45, 656-667
27. Subbiah, M., *et al.* (2011) Selection Pressure Required for Long-Term Persistence of bla(CMY-2)-Positive IncA/C Plasmids. *Appl Environ Microbiol* 77, 4486-4493
28. Smith, J. (2010) Superinfection drives virulence evolution in experimental populations of bacteria and plasmids. *Evolution* 65, 831-841
29. Modi, R.I., *et al.* (1992) Genetic changes accompanying increased fitness in evolving populations of *Escherichic coli*. *Genetics* 130, 241-249
30. Bouma, J.E., and Lenski, R.E. (1988) Evolution of a bacteria plasmid association. *Nature* 335, 351-352

31. De Gelder, L.*, et al.* (2008) Adaptive plasmid evolution results in host-range expansion of a broad-host-range plasmid. *Genetics* 178, 2179-2190

32. Dionisio, F.*, et al.* (2005) The evolution of a conjugative plasmid and its ability to increase bacterial fitness. *Biology Letters* 1, 250-252

33. Heuer, H.*, et al.* (2007) Frequent conjugative transfer accelerates adaptation of a broad-host-range plasmid to an unfavorable Pseudomonas putida host. *FEMS Microbiol Ecol* 59, 738-748

34. Turner, P.E.*, et al.* (1998) Tradeoff between horizontal and vertical modes of transmission in bacterial plasmids. *Evolution* 52, 315-329

35. Scott, M.*, et al.* (2010) Interdependence of Cell Growth and Gene Expression: Origins and Consequences. *Science* 330, 1099-1102

36. Stoebel, D.M.*, et al.* (2008) The cost of expression of Escherichia coli lac operon proteins is in the process, not in the products. *Genetics* 178, 1653-1660

37. Torres, E.M.*, et al.* (2007) Effects of aneuploidy on cellular physiology and cell division in haploid yeast. *Science* 317, 916-924

38. Harr, B., and Schlotterer, C. (2006) Gene expression analysis indicates extensive genotype-specific crosstalk between the conjugative F-plasmid and the E-coli chromosome. *BMC Microbiol* 6, 7

39. Mc Ginty, S.É., and Rankin, D.J. (2012) The evolution of conflict resolution between plasmids and their bacterial hosts. *Evolution* Early view

40. Sota, M.*, et al.* (2010) Shifts in the host range of a promiscuous plasmid through parallel evolution of its replication initiation protein. *Isme Journal* 4, 1568-1580

41. Norman, A.*, et al.* (2009) Conjugative plasmids: vessels of the communal gene pool. *Philosophical Transactions of the Royal Society B-Biological Sciences* 364, 2275-2289

42. Thompson, J.N. (2005) *The Geographic Mosaic of Coevolution*. The University of Chicago Press, Chicago, USA.